EPA Response to Mixture Testing with Tetra Tech Follow-up

a. The tested concentration of aluminum was 750 ug/L, which apparently is the Alaska state acute water quality standard. The rational for using the highest possible concentration of aluminum in the mixture test is questionable at best. The concentration of aluminum used in the test is important because the investigators acknowledge that aluminum (or alum) is a standard flocculant in wastewater treatment and readily combines with other metals, resulting in low dissolved metals in solution.

Because no acute site-specific aluminum standard is proposed and a chronic site-specific standard is proposed and acute testing was used in the WER study, aluminum at the state acute standard concentration (750 $\mu g/L$ goal) was included to represent a worst-case scenario. EPA's statement is contradictory. We agree that aluminum may form a floc that could interact with other toxicants. However, EPA appears to suggest that aluminum should be viewed only as a potential ameliorating factor and not as a toxic pollutant. This is not in keeping with standard application of 304(a) water quality criteria.

b. The percent survival of 85% obtained in the mixture test with D. magna is below the 90% survival that is the standard for acceptability of acute toxicity tests. It appears that because the percent survival was less than 90%, the standard for acceptability was inappropriately changed to one based on hypothesis testing. Satisfying a standard for acceptability of 90% is not unreasonable because the survival percentages in the lab control and the site water were 95% and 100%, respectively. The percent survival in the mixture test (i.e., the spiked site water test) was too low.

The site water treatment spiked with metals was observed to result in 85% survival of test organisms. The 90% survival test acceptability criterion is only applicable to control treatments (and was satisfied in this study). The goal of the study was to evaluate the differential survival of *Daphnia magna* exposed to the control and site water at selected metal concentrations. Thus, the hypothesis test was used to demonstrate that no significant difference in survival was observed.

c. For copper, lead, and zinc, dissolved metal is the only metal that can be considered bioavailable and toxic in this mixture test. Thus the concentrations of these three metals that can be deemed safe in the mixture test are those given in Tables 2 and 3 in the columns labeled "Measured dissolved". It should be acceptable to the use the averages of the concentrations given in the two tables for each of the three metals. These concentrations are about 42, 13, and 54% of the proposed dissolved acute criteria for these three metals. Therefore, the mixture test shows that the toxicity of the mixture of the three metals is greater than what would be predicted on the basis of tests on the three metals individually, especially considering that the percent survival in the mixture test with D.

magna was only 85%.

EPA incorrectly states that the dissolved concentrations of copper, lead, and zinc are the only ones that may be considered bioavailable and toxic. It is within the authority of Alaska to base standards on total recoverable concentrations. Is PacRim requesting site-specific acute standards?

The goal of this test was to evaluate the toxicity of a metal mixture based on WER-derived site-specific criteria. Achieving a test solution with each of these three metals (Cu, Pb, Zn) at the proposed dissolved criteria concentrations is not possible in site water without dramatically lowering the pH. Thus, the threshold required by EPA to demonstrate "no effect" is unrealistic. A testing solution in which the total concentrations were at or above site-specific standards is likely the best case testing scenario.

More can be said here about the testing solution being representative of the natural condition.

d. It is inappropriate to convert the dissolved concentrations from the mixture test to total concentrations for use in site-specific criteria using the percent dissolved observed in the mixture test because the percent dissolved in the mixture test is greatly affected by the high concentration of aluminum that was present in that test. The values that are used for percent dissolved need to be consistent not only with the concentration of aluminum in the downstream site water but also with the form of aluminum in the downstream site water. Alternatively, the values of percent dissolved obtained from the tests on the individual metals can be used.

See comments on selected Al concentrations above. Specifically, Al concentrations represent a worst-case scenario. Additional toxicant concentration should not be considered beneficial, correct?

e. It would be interesting to see what results would be produced by a mixture test in which no aluminum was added. It is very possible that the ratios of the dissolved concentrations of the three metals would be different from the ratios that were produced by the mixture test that was performed using a high concentration of aluminum.

Possibly, but this is not the testing scenario suggested in the WER guidance document, nor was it suggested by EPA during discussions which led up to this testing.

f. Near the bottom of page 2 of the 1-7-11 memo from Diamond and Latimer to Graham, it says that dissolved metal "is bound to natural constituents in the site water and is no longer measurable as dissolved" and "this is especially the case for lead but is true for

copper and zinc as well". However, "bound to natural constituents" is not necessarily true if a high concentration of aluminum is present in the mixture test.

As Tetra Tech understands it, the presence of aluminum in the testing solutions is required by WER guidance and EPA suggestion. Further, the presence of aluminum would not prevent binding of metals to the naturally occurring constituents in the site water.

g. The last sentence on page 6 ends by saying "these results demonstrate that the proposed criteria are suitable for application to this system", but this is not true for the proposed dissolved criteria. This would not even be true for the proposed dissolved criteria if the percent survival in the mixture test with D. magna was 95% rather than 85%. The mixture test with D. magna demonstrates that a mixture of the proposed dissolved criteria for copper, lead, and zinc is not suitable for application to this system.

Tetra Tech disagrees with this statement. Please see response to comment b, above for discussion of the survival of D. magna in the site solution. The threshold of demonstration being required in this instance is not required of national criteria applied as state standards which are always considered on a single-compound basis. Additionally, such mixture testing is rarely if ever required in development of other site-specific criteria for multiple metals. Tetra Tech is not familiar with instances of this type of mixture testing being required and the only example EPA R10 could provide was as part of a Recalculation Study and not a WER study. Finally, as discussed above and prior to testing, developing a solution with the selected concentrations of the dissolved fractions of Cu, Pb, and Zn in the natural water is likely impossible with or without addition of aluminum.